ELSEVIER

Biochimica et Biophysica Acta 1500 (2000) 297–305

BIOCHIMICA ET BIOPHYSICA ACTA

BBA

www.elsevier.com/locate/bba

Alterations of Na^+ homeostasis in hepatocyte reoxygenation injury

Rita Carini, Maria Grazia De Cesaris, Roberta Splendore, Marco Bagnati, Giorgio Bellomo, Emanuele Albano *

Department of Medical Science, University 'A. Avogadro' of East Piedmont, Via Solaroli 17, 27100 Novara, Italy

Received 12 July 1999; received in revised form 11 November 1999; accepted 20 December 1999

Abstract

Reperfusion injury represents an important cause of primary graft non-function during liver transplantation. However, the mechanism responsible for cellular damage during reoxygenation has not yet been completely understood. We have investigated whether changes in intracellular Na^+ distribution might contribute to cause hepatocyte damage during reoxygenation buffer after 24 h of cold storage. Hepatocyte reoxygenation resulted in a rapid increase in cellular Na^+ content that was associated with cytotoxicity. Na^+ accumulation and hepatocyte death were prevented by the omission of Na^+ from the incubation medium, but not by the addition of antioxidants. Blocking Na^+/H^+ exchanger and Na^+/HCO_3^- co-transporter by, respectively, 5-(*N,N*-dimethyl)-amiloride or omitting HCO_3^- from the reoxygenation medium significantly decreased Na^+ overload and cytotoxicity. Stimulation of ATP re-synthesis by the addition of fructose also lowered Na^+ accumulation and cell death during reoxygenation. A significant protection against Na^+-mediated reoxygenation injury was evident in hepatocytes maintained in an acidic buffer (pH 6.5) or in the presence of glycine. The cytoprotective action of glycine or of the acidic buffer was reverted by promoting Na^+ influx with the Na^+/H^+ ionophore monensin. Altogether, these results suggest that Na^+ accumulation during the early phases of reoxygenation might contribute to liver graft reperfusion injury.

Keywords: Sodium; Reperfusion injury; Liver transplantation; Glycine; Acidic pH; Cold storage

1. Introduction

Poor organ preservation and reperfusion injury still represent an important cause of primary liver non-function following orthotopic transplantation [1]. The contribution of reperfusion injury in causing liver graft failure has been a matter of extensive investigations that have demonstrated the involvement of oxygen radicals, nitric oxide, cytokines, eicosanoids and proteases in causing hepatocyte and endothelial cells damage during liver reperfusion [1–3].

Recent data have implicated ionic disturbances within the myocardium as a critical factor in the pathogenesis of heart damage during ischemia/reperfusion [4]. In particular, it has been observed that the activation of cardiac sarcolemmal membrane Na^+/H^+ exchanger in response to intracellular acidosis developing during the anoxic period promotes a large influx of Na^+ [4]. Indeed, Na^+/H^+ exchanger inhibitors as well as heart perfusion with either low Na^+-

* Corresponding author. Fax: +39 (0321) 620421; E-mail: albano@med.no.unipmn.it

0925-4439/00/$ – see front matter
PII: S0925-4439(99)00114-3

containing solutions or acidic buffers reduce myocardial reperfusion injury [4,5]. The increase in myocardiocyte Na^+ content occurring during reoxygenation can only partially be controlled by the activity of Na^+/K^+ ATPase [4] and leads to the reverse activation of Na^+/Ca^{2+} exchanger, thus inducing an elevation of cytosolic Ca^{2+} levels [4,5]. Elevated cytosolic Na^+ and Ca^{2+} levels are then responsible for causing myocardial arrhythmia and hypercontractions that represent the main complication of heart reperfusion [5].

We have previously reported that ATP depletion and cellular acidosis occurring in isolated rat hepatocytes exposed to oxidative stress, mitochondrial inhibitors or hypoxia are followed by an increase in intracellular Na^+ levels [6,7]. In these conditions, the combined activation of Na^+/H^+ exchanger and of Na^+/HCO_3^- co-transporter in response to cytosolic acidification and the block of ATP-dependent Na^+ efflux through the Na^+/K^+ ATPase resulted in Na^+ accumulation [7]. Na^+ overload played a critical role in the development of irreversible cell injury and preventing Na^+ accumulation by hepatocyte incubation in a Na^+-free buffer or in an acidic medium (pH 6.5) greatly reduced cell killing [6,7]. Parallel studies by Fiegen and colleagues have confirmed the accumulation of Na^+ in isolated perfused rat livers exposed to warm ischemia and have demonstrated the beneficial effect of furesimide or bumetamide, two inhibitors of the Na^+-K^+-$2Cl^-$ co-transporter, in preventing hypoxic liver injury [8]. Intracellular acidosis and depletion of ATP stores are common features during cold preservation of explanted livers [1]. Thus, we have investigated the possibility that perturbations of Na^+ homeostasis might contribute to liver damage during the early phase of reperfusion.

Alterations in ion distribution are difficult to study following allograph organ transplantation in animals or during reperfusion of isolated organs. In this study, the changes in Na^+ homeostasis during reoxygenation were investigated using isolated rat hepatocytes cold-stored 24 h in University of Wisconsin (UW) solution and subsequently transferred in an oxygenated Na^+-containing buffer in order to mimic the changes in extracellular ion composition that liver grafts face during the early phases of reperfusion.

2. Materials and methods

Collagenase (type I), *N*-(2-hydroxyethyl)-piperazine-*N*′-(2-ethanesulfonic acid) (HEPES), 2-(*N*-morpholino)-ethanesulfonic acid (MES), propidium iodide (PI), L-glycine and monensin were purchased from Sigma (St. Louis, MO, USA). 5-(*N,N*-Dimethyl)-amiloride (DMA) was obtained from Calbiochem–Novabiochem (San Diego, CA, USA). Percoll was supplied by Pharmacia (Uppsala, Sweden). All the other chemicals were of analytical grade and were purchased from Merck (Darmstad, Germany).

Male Wistar rats (180–250 g weight) were obtained from Nossan (Corezzana, Italy) and allowed free access to water and food. The use and care of the animals were approved by the Italian Ministry of Health and by the University Commission for Animal Care following the criteria of the Italian National Research Council. Isolated rat hepatocytes were prepared by liver perfusion with collagenase as previously described [7]. Cell viability, estimated at the beginning of the experiments, ranged between 85 and 90%. For the experiments, isolated rat hepatocytes (5×10^6 cells/ml) were stored for 24 h at 4°C in UW solution (Dupont, Wilmington, DE, USA) under a nitrogen atmosphere. After re-warming at room temperature in UW solution, the cells were separated by centrifugation, re-suspended at the concentration of 1×10^6 cells/ml in differently modified Krebs–Henseleit media equilibrated with 95% O_2-5% CO_2 and further incubated at 37°C. The basic incubation medium consisted of a Krebs–Henseleit–HEPES (KHH) buffer containing 118 mmol/l NaCl, 4.7 mmol/l KCl, 1.2 mmol/l KH_2PO_4, 1.3 mmol/l $CaCl_2$, 25 mmol/l $NaHCO_3$ and 20 mmol/l HEPES at pH 7.4. For the experiments performed in the absence of Na^+, NaCl and $NaHCO_3$ were replaced by 118 mmol/l choline chloride and 25 mmol/l $KHCO_3$, respectively. The bicarbonate-free buffer contained 143 mmol/l NaCl and was fluxed with 95% O_2, 5% N_2 gas mixture. In the acidic Krebs–Henseleit buffer, HEPES was replaced by 20 mmol/l MES and the pH was adjusted to 6.5 with HCl. Modified KHH buffer was also prepared by using LiCl instead of NaCl or by substituting Na^+ for K^+.

Cell viability was estimated by microscope-counting the hepatocytes excluding trypan blue or by flow cytometric determination of nuclear fluorescence

after the addition of PI. For flow cytometry assay, 1 ml aliquots of the cell suspensions were taken and diluted 10 times in the respective incubation buffer containing 180 μg/ml PI and immediately analyzed with a FACScan analyzer (Becton Dickinson, San Jose, CA, USA) tuned to 488 nm and delivering 15 mW output power. The red fluorescence of PI was measured through a 585 nm band-pass filter. The sheath medium was Diluid (J.T. Baker B.V., Deventer, The Netherlands) and the flow rate was optimized in order to allow fast sample acquisition and data reproducibility. Ten thousand hepatocytes were analyzed for each sample and the values were expressed as percent of the total cell number.

Intracellular Na^+ levels were measured in viable cells separated by centrifugation through 3 ml of Percoll solution ($d=1.06$) in 0.25 mol/l sucrose as previously reported [7]. After centrifugation, the Percoll solution was rapidly removed by aspiration, and the cell pellets were extracted with 0.5 ml of 0.8 N perchloric acid. Na^+ was measured in aliquots of the protein-free acidic supernatants diluted 200 times with distilled water using a Varian AA-1475 atomic absorption spectrophotometer (Varian Instruments Div., Palo Alto, CA, USA). The values were corrected for the protein content of each pellet. Na^+ concentration was expressed in mmol/l considering that 1×10^6 cells correspond to 1.8 mg of protein and that hepatocytes in suspension can be assimilated to spheres with an average diameter of 20 μm [9]. In some experiments, the changes in the intracellular Na^+ concentration were also monitored by using the fluorescent Na^+ probe sodium binding benzofuran isophthalate (SBFI) [10]. Isolated hepatocytes (3×10^6 cells/ml) maintained 24 h at 4°C in UW solution were re-warmed at 25°C and incubated 60 min at 25°C with SBFI-acetoxymethyl ester (10 μmol/l final concentration in DMSO) directly added to the UW solution. After incubation, the cells were separated by centrifugation and re-suspended in oxygenated complete KHH buffer or Na^+-free KHH buffer. The changes in SBFI fluorescence were monitored during a subsequent 30 min incubation using a Hitachi 4500 spectrofluorimeter set alternatively at the 340 and 385 nm excitation wavelengths and fluorescence was recorded at 490 nm wavelength. The ratio of fluorescence values obtained with 340 nm and 385 nm excitation were calculated after correction for spontaneous SBFI fluorescence. Calibration of SBFI fluorescence was performed by addition of hepatocytes to solutions of known Na^+ concentration following the addition of the Na^+ ionophore gramicidin D (2 μmol/l) [10].

Hepatocyte ATP was determined by reverse-phase high performance liquid chromatography, after protein precipitation with perchloric acid, using a Hibar Lichrospher 100RP-18 column (Merck, Darmstad, Germany) and 0.1 M potassium phosphate buffer pH 5.3 as eluent [7].

Lipid peroxidation was estimated by measuring malondialdehyde (MDA) accumulation in protein-free supernatant as previously reported [11].

Statistical analysis for multiple comparisons was performed by the one-way ANOVA test with Bonferroni's corrections. The distribution normality of the groups considered was preliminary evaluated by the Shapiro–Wilk test.

3. Results

Twenty-four hours of cold storage in UW solution did not appreciably affect the viability of isolated hepatocytes and, after re-warming, about 90% of the cells excluded trypan blue and PI. The intracellular Na^+ content was not modified by the incubation in cold UW solution (13 ± 3.1 mmol/l as compared to 14.2 ± 1.9 mmol/l in freshly isolated cells). Upon reoxygenation in KHH medium at pH 7.4, hepatocytes displayed a rapid and progressive increase of the intracellular Na^+ levels, as demonstrated by the use of either the fluorescent sodium probe SBFI or by the direct measurement of Na^+ content in viable cells by atomic adsorption (Fig. 1). Elevation of hepatocyte Na^+ content was appreciable already after 5 min from reoxygenation and preceded the decline of cell viability (Fig. 2). After 15 min reoxygenation, oxidative damage was detectable by the accumulation of MDA (0.88 ± 0.20 nmol/10^6 cells as compared to 0.16 ± 0.04 nmol/10^6 cells before reoxygenation). The addition of the antioxidant *N,N′*-phenyl-phenylenediamine (DPPD) (5 μmol/l) to block oxidative damage completely prevented MDA formation (0.12 ± 0.07 nmol/10^6 cells), but did not reduce Na^+ accumulation and cell death (Figs. 1 and 2). The development of irreversible cell

injury was, instead, prevented when hepatocytes were reoxygenated in Na^+-free KHH medium, which abolished Na^+ overload (Fig. 1). Substitution of K^+ for Na^+ in the reoxygenation buffer also prevented hepatocyte killing, whereas cytotoxicity developed when hepatocytes were reoxygenated in KHH buffer where Na^+ was substituted by Li^+ (Fig. 2). Li^+, but not K^+, can replace Na^+ for the activity of membrane ion transport systems involved in cellular pH buffering [12]. This suggested that hepatocyte killing during reoxygenation might be promoted by ionic unbalances consequent to the activation of pH regulatory systems.

A decrease of intracellular pH is a common feature of anoxic tissues and cytosolic acidosis has been observed during liver cold preservation [13]. In hepatocytes, the acid buffering system relies on the activity of Na^+/H^+ exchanger and Na^+/HCO_3^- co-transporter located in the basolateral membranes [12]. Blocking plasma membrane Na^+/H^+ exchanger with 10 μmol/l DMA or interfering with the activity of Na^+-HCO_3^- co-transporter by using a bicarbonate-free incubation buffer significantly reduced intracellular Na^+ accumulation and hepatocyte reoxygenation injury (Fig. 3). This indicated that Na^+ inflow during reoxygenation was mediated by the activation of acid buffering systems in response to the intracellular acidosis that developed during cold anoxia. Nonetheless, the impairment of Na^+ extrusion by plasma membrane Na^+/K^+ ATPase might also contribute to Na^+ accumulation. Hepatocyte ATP levels at the end of 24 h cold storage were very low (1.76 ± 0.52 nmol/10^6 cells) as compared to those of freshly isolated cells (12.4 ± 1.22 nmol/10^6 cells) and only slowly recovered upon reoxygenation. Addition of 10 mmol/l fructose to the reoxygenation buffer allowed a more efficient recovery of intracellular ATP (5.4 ± 0.74 nmol/10^6 cells after 60 min reoxyge-

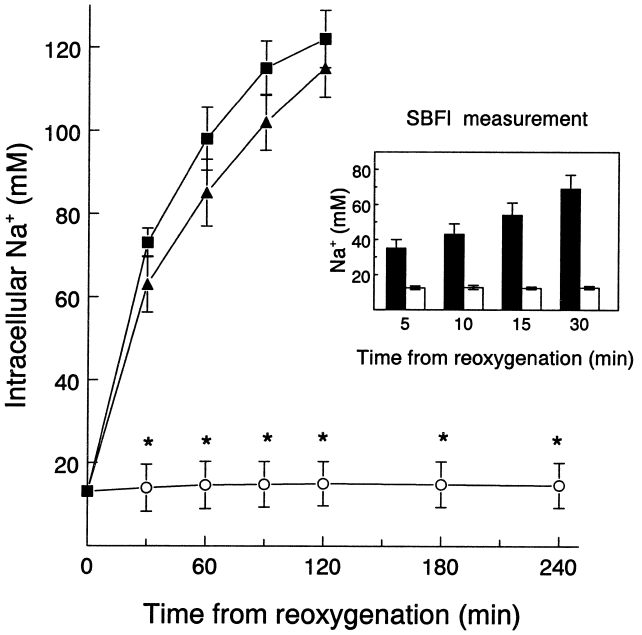

Fig. 1. Changes in intracellular Na^+ content during the reoxygenation of isolated rat hepatocytes cold-stored 24 h at 4°C in UW solution. After cold storage, the hepatocytes were reoxygenated as described in Section 2 and incubated at 37°C in complete KHH buffer (▲); in KHH buffer containing 5 μmol/l DPPD (■); in a Na^+-free KHH buffer (○). The insert shows the measurement of intracellular Na^+ by the fluorescent Na^+ indicator SBFI in hepatocytes reoxygenated in complete (filled bars) or Na^+-free (open bars) KHH buffers. The results are means of 4–6 different experiments ± S.D. Statistical significance: * $P < 0.001$ versus hepatocytes reoxygenated in complete KHH buffer.

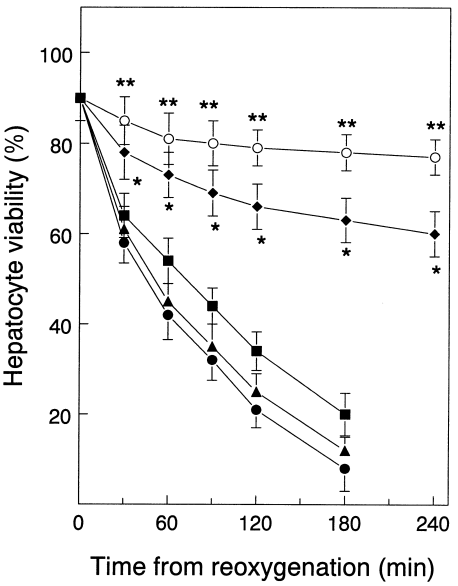

Fig. 2. Changes in cell viability during the reoxygenation of isolated rat hepatocytes cold-stored 24 h at 4°C in UW solution. After cold storage, the hepatocytes were reoxygenated as described in Section 2 and incubated at 37°C in complete KHH buffer (▲); in KHH buffer containing 5 μmol/l DPPD (●); in a Na^+-free KHH buffer (○); in a modified KHH buffer containing 4.7 mmol/l Na^+ and 143 mmol/l K^+ (◆); in KHH buffer where NaCl was substituted by an equimolar amount of LiCl (■). The results are means of 4–6 different experiments ± S.D. Statistical significance: * $P < 0.01$ or ** $P < 0.001$ versus hepatocytes reoxygenated in complete KHH buffer.

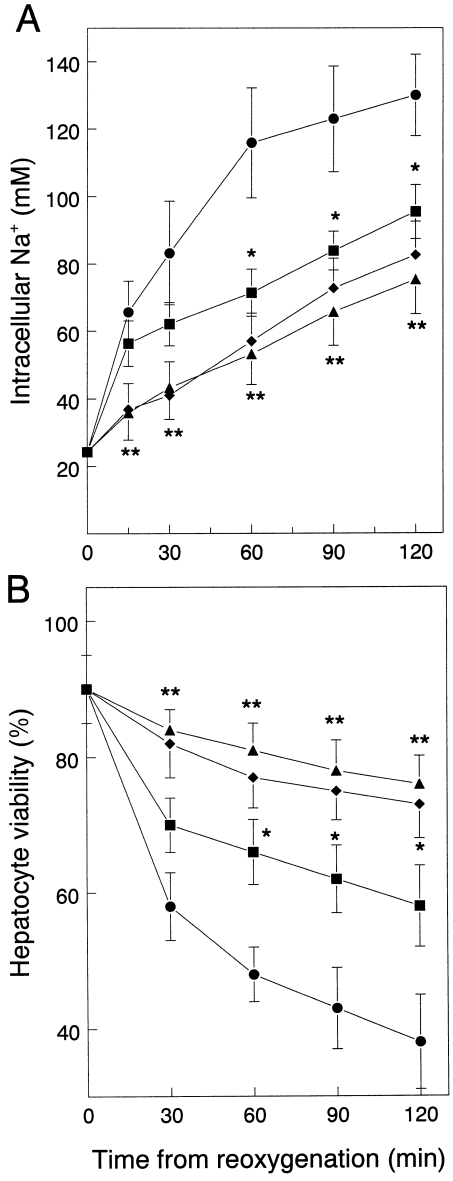

Fig. 3. Effect of hepatocyte acid buffering system inhibition and of fructose supplementation on the intracellular Na^+ content (A) and cell viability (B) during the reoxygenation of isolated rat hepatocytes cold-stored 24 h at 4°C in UW solution. After cold storage, the hepatocytes were reoxygenated as described in Section 2 and incubated at 37°C in complete KHH buffer (●); in KHH buffer containing 10 μmol/l DMA (▲); in HCO_3^--free KHH buffer (♦); in KHH buffer containing 10 mmol/l fructose (■). The results are means of 3–4 different experiments ± S.D. Statistical significance: * $P < 0.01$ and ** $P < 0.001$ versus hepatocytes reoxygenated in complete KHH buffer.

nation). In the presence of fructose, the increase of intracellular Na^+ and the development of cytotoxicity during reoxygenation of hepatocytes were appreciably lowered (Fig. 3).

Upon reperfusion, the intracellular pH is rapidly restored to physiological values. However, it has been reported that the recovery of intracellular pH during reperfusion precipitates cell injury [14]. This effect, also known as 'pH paradox', has been observed in several tissues [14–16] and can be prevented by lowering the pH of the extracellular fluids [5,17,18]. Fig. 4 demonstrates that the cytoprotective effect exerted by hepatocyte reoxygenation in acidic Krebs–Henseleit medium (pH 6.5) was associated with a significant inhibition of Na^+ accumulation. Promoting Na^+ influx by the addition of the Na^+/H^+ ionophore monensin (10 μmol/l) abolished the protective action of the acidic buffer (Fig. 4). This suggested that extracellular acidosis might reduce hepatocyte reoxygenation injury by interfering with Na^+ accumulation.

Recent studies have demonstrated that the addition of glycine also improves reperfusion injury in perfused rat liver and in isolated endothelial cells [19,20]. Thus, further experiments were performed to investigate whether glycine supplementation might attenuate Na^+-mediated hepatocyte reperfusion injury. As shown in Fig. 4, addition of glycine (2 mmol/l) completely prevented hepatocyte killing during reoxygenation. Glycine also blocked Na^+ accumulation developing upon transferring the cells in oxygenated Na^+-containing buffer (Fig. 4). The protection exerted by glycine was, however, completely lost in the presence of monensin (Fig. 4), indicating that interference with Na^+ might account for the cytoprotective action of glycine.

4. Discussion

Liver reperfusion injury is the result of a complex series of events that include the formation of oxygen and nitrogen free radicals, the impairment of endothelial cell functions, the recruitment and the activation of phagocytes and the release of cytokines [1–3]. Less attention has been paid to the possible role of alterations in ion distribution that might occur in the early phase of hepatic graft reperfusion. Just before

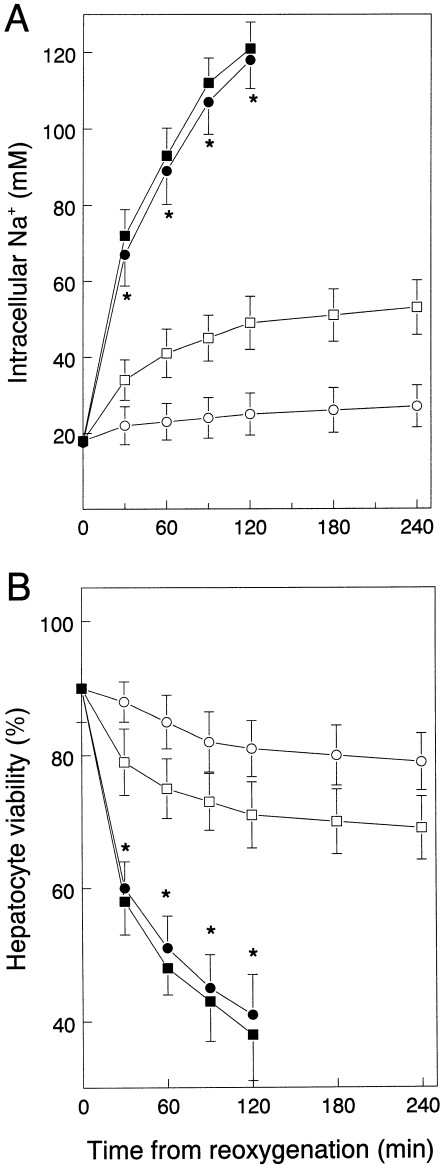

Fig. 4. Effect of glycine and of the incubation in an acidic medium (pH 6.5) on intracellular Na^+ content (A) and cell viability (B) during the reoxygenation of isolated rat hepatocytes cold-stored 24 h at 4°C in UW solution. After cold storage, the hepatocytes were reoxygenated as described in Section 2. The hepatocytes were then incubated at 37°C under the following conditions: (○) KHH buffer pH 7.4 containing 2 mmol/l glycine; (●) KHH buffer pH 7.4 plus glycine and 10 μmol/l monensin; (□) Krebs–Henseleit buffer pH 6.5; (■) Krebs–Henseleit buffer pH 6.5 plus monensin. The results are means of three different experiments ± S.D. Statistical significance: * $P < 0.001$ versus hepatocytes incubated without monensin.
←

implantation, the livers are, in fact, currently rinsed with Ringer's solution in order to remove high K^+ concentrations of UW solution that could cause cardiac arrhythmia. During this procedure, liver cells are exposed to reoxygenation as well as to a rapid change in the Na^+ and K^+ content of the extracellular fluids since Ringer's solution contains 130 mmol/l Na^+ and 4 mmol/l K^+ as compared to 25 mmol/l Na^+ and 115 mmol/l K^+ of the UW solution.

To investigate whether alterations of Na^+ homeostasis might have a role in the pathogenesis of liver reoxygenation injury, we have used isolated rat hepatocytes cold-stored 24 h in UW solution and subsequently transferred to an oxygenated Na^+-containing buffer in order to mimic the changes in extracellular ion composition that liver grafts face during the early phases of reperfusion. The results obtained demonstrated that: (i) intracellular Na^+ increased very early upon reoxygenation; (ii) oxidative damage was not involved in causing Na^+ influx; (iii) Na^+ inflow was likely attributed to the activation of acid buffering systems involving Na^+/H^+ exchanger and Na^+/HCO_3^- co-transporter in response to intracellular acidosis; (iv) preventing Na^+ accumulation greatly decreased hepatocyte killing during reoxygenation.

Forestal and co-workers [21] have recently reported that hepatocyte cold storage in UW solution disturbs pH regulatory mechanisms by lowering Na^+/H^+ exchange activity and by enhancing Na^+/HCO_3^- co-transport. Nonetheless, the overall pH buffering capacity of hepatocytes does not appear to be affected [21]. The role of acid buffering systems in triggering Na^+ influx during reoxygenation is consistent with recent observations concerning the cardioprotective action of Na^+/H^+ exchanger inhibitors on heart reperfusion damage [22–24]. In the early phases of reperfusion, when cellular energy stores are still low, the reduced availability of ATP might also affect the activity of plasma membrane Na^+/K^+-translocase, thus contributing to Na^+ accumulation. Indeed, stimulation of ATP re-synthesis by the addition of fructose to the reoxygenation medium reduced Na^+ overload and improved hepatocyte survival during reoxygenation.

Little is known about the mechanisms responsible for Na^+-mediated hepatocyte injury during reoxygenation. Serrar and Haddad have recently reported that cold preservation in UW solution affects the

hepatocyte volume regulatory capacity [25]. We have shown that Na^+ overload caused cell swelling in ATP-depleted hepatocytes [6] and that the impairment of volume regulatory mechanisms was critical for the development of hepatocyte necrosis induced by mitochondrial inhibition or oxidative stress [26]. Therefore, it is possible that osmotic stress due to Na^+ accumulation might contribute to cell injury during reoxygenation. However, the contribution of Ca^{2+}-dependent mechanisms cannot be excluded [27], since Na^+ increase promotes the elevation of cytosolic Ca^{2+} levels through the reversed activation of Na^+/Ca^{2+} exchanger [28,29].

In recent years, the beneficial effects of liver reoxygenation under acidic conditions have been exploited by the development of Carolina Rinse solution that, among other features, is characterized by an acidic pH (6.5) [30,31]. Carolina Rinse solution has been suggested to protect against hepatic reperfusion injury by suppressing Kupffer cell activation, by inhibiting free radical formation and by decreasing sinusoid endothelial cell killing [3,20]. Furthermore, recent data demonstrate that acidic incubation medium prevents the onset of mitochondrial permeability transition in cultured hepatocytes exposed to hypoxia/reoxygenation [32]. We propose that the use of an acidic rinse solution might reduce liver reperfusion damage also by avoiding Na^+ accumulation within liver cells during the washout of UW solution. The mechanism by which acidic extracellular pH reduces Na^+ influx during reperfusion has not been investigated in detail, but likely involves interference with cellular acid buffering systems [7]. The inhibition of Na^+ influx by acidic pH might be important for the preservation of liver sinusoid endothelial cells by Carolina Rinse solution [20], since post-ischemic activation of Na^+/H^+ exchanger has been shown to trigger lung microvascular injury during reperfusion [18].

The addition of glycine has been reported to protect isolated renal tubular cells and hepatocytes from ischemia, ATP depletion and cold storage injury [33–35]. Moreover, glycine reduces warm reperfusion injury in perfused rat livers [19,36] and improves survival and post-operative liver injury after orthotopic liver transplantation in rats [37]. The effect of glycine has been ascribed to the prevention of reperfusion-induced killing of sinusoidal endothelial cells [20]. The present results demonstrate that the inhibition of Na^+ accumulation during reoxygenation might also contribute to the cytoprotective action of glycine on liver reperfusion damage. The action of glycine on Na^+ influx is likely mediated by the block of specific Cl^- channels, since the glycine receptor antagonist, strychnine, and the incubation in a Cl^--free medium similarly protected against Na^+ overload and hepatocyte killing during warm hypoxia or KCN poisoning [38].

In conclusion, the results presented indicate that an increase of intracellular Na^+ can be involved in liver cell injury during the early phases of reoxygenation and suggest that treatments able to reduce Na^+ accumulation might be usefully employed in the prevention of liver graft reoxygenation damage.

Acknowledgements

This work has been supported by the Ministry of University and Scientific and Technologic Research, Rome (Project: Patologia da Radicali Liberi e degli Equilibri Redox) and by The Consorzio Interuniversitario Biotecnologie (Project: Biotecnologie nel Trapianto Epatico).

References

[1] P.A. Clavien, P.R.C. Harvey, S.M. Strasberg, Preservation and reperfusion injuries in liver allografts: An overview and synthesis of current studies, Transplantation 53 (1992) 957–958.
[2] H. Jaeschke, Preservation injury: mechanisms, prevention and consequences, J. Hepatol. 25 (1996) 774–780.
[3] J.J. Lemasters, R.G. Thurman, Reperfusion injury after liver preservation for transplantation, Annu. Rev. Pharmacol. Toxicol. 37 (1997) 327–328.
[4] G.N. Pierce, M.P. Czubryt, The contribution of ion imbalance to ischemia/reperfusion-induced injury, J. Mol. Cell Cardiol. 27 (1995) 53–63.
[5] L.M. Buja, Modulation of myocardial response to ischemia, Lab. Invest. 78 (1998) 1345–1373.
[6] R. Carini, R. Autelli, G. Bellomo, M.U. Dianzani, E. Albano, Sodium-mediated cell swelling is associated with irreversible damage of isolates hepatocytes exposed to hypoxia or mitochondrial toxins, Biochem. Biophys. Res. Commun. 206 (1995) 180–185.
[7] R. Carini, G. Bellomo, A. Benedetti, R. Fulceri, A. Gamberucci, M. Parola, M.U. Dianzani, E. Albano, Alteration of

Na^+ homeostasis as a critical step in the development of irreversible hepatocyte injury after adenosine triphosphate depletion, Hepatology 21 (1995) 1089–1098.
[8] R.J. Fiegen, U. Rauen, M. Hartmann, U.K.M. Deking, H. DeGroot, Decrease of ischemic injury to the isolated perfused rat liver by loop diuretics, Hepatology 25 (1997) 1425–1431.
[9] M. Borin, W. Siffert, Stimulation by trombin increases the cytosolic free Na^+ concentration in human platelets. Studies with the novel fluorescent cytosolic Na^+ indicator sodium binding benzofuran isophthalate, J. Biol. Chem. 265 (1990) 19543–19560.
[10] W. Pfaller, C. Willinger, B. Stoll, C. Hallbrucker, F. Lang, D. Häussinger, Structural reaction pattern of hepatocytes following exposure to hypotonicity, J. Cell. Physiol. 154 (1993) 248–253.
[11] P. Clot, M. Tabone, S. Aricò, E. Albano, Monitoring oxidative damage in patients with liver cirrhosis and different daily alcohol intake, Gut 35 (1994) 1637–1643.
[12] J.L. Boyer, J. Graf, P.J. Meier, Hepatic transport systems regulating pH, cell volume, and bile secretion, Annu. Rev. Physiol. 54 (1992) 415–438.
[13] L. Rossaro, N. Murase, C. Caldwell, F. Hassan, A. Casavilla, T.E. Starzl, C. Ho, D.H. VanThiel, Phosphorus 31-nuclear magnetic resonance spectroscopy of rat liver during simple storage of continuous hypothermic perfusion, J. Lab. Clin. Med. 120 (1992) 559–568.
[14] G.J. Gores, A.L. Nieminen, B.E. Wray, B. Herman, J.J. Lemasters, Intracellular pH during 'chemical hypoxia' in cultured rat hepatocytes. Protection by intracellular acidosis against the onset of cell death, J. Clin. Invest. 83 (1989) 386–396.
[15] S.F. Bronk, G.J. Gores, Acidosis protects against lethal oxidative injury of liver sinusoidal endothelial cells, Hepatology 14 (1991) 150–157.
[16] J.M. Bond, I.S. Harper, E. Chacon, I.M. Reece, B. Herman, J.J. Lemasters, The pH paradox in the pathophysiology of reperfusion injury to neonatal cardiac myocytes, Ann. N.Y. Acad. Sci. 732 (1994) 25–37.
[17] R.T. Currin, G.J. Gores, R.G. Thurman, J.J. Lemasters, Protection by acidotic pH against anoxic cell killing in perfused rat liver: evidence for a pH paradox, FASEB J. 5 (1991) 207–210.
[18] T.M. Moore, P.L. Khimenko, A.E. Taylor, Restoration of normal pH triggers ischemia-reperfusion injury in lung by Na^+/H^+ exchange activation, Am. J. Physiol. 259 (1995) H1501–H1505.
[19] Z. Zhong, S. Jones, R.G. Thurman, Glycine minimizes reperfusion injury in a low-flow reflow liver perfusion model in the rat, Am. J. Physiol. 270 (1996) G332–G338.
[20] T. Currin, J.C. Caldwell-Kenkel, S.N. Lichtman, S. Bachmann, Y. Takei, S. Kawano, R.G. Thurman, J.J. Lemasters, Protection by Carolina rinse solution, acidotic pH, and glycine against lethal reperfusion injury to sinusoidal endothelial cells of rat livers stored for transplantation, Transplantation 62 (1996) 1549–1558.
[21] D.A. Forestal, J. Haimovici, P. Haddad, Different effect of cold storage and rewarming on three pH regulating transporters in isolated rat hepatocytes, Am. J. Physiol. 272 (1997) G638–G645.
[22] Y.V. Ladilov, B. Siegmund, H.M. Piper, Protection of reoxygenated cardiomyocytes against hypercontracture by inhibition of Na^+/H^+ exchange, Am. J. Physiol. 268 (1995) H1531–H1539.
[23] T. Miura, T. Ogawa, K. Sozuki, M. Goto, K. Shimamoto, Infarct size limitation by a new Na^+-H^+ exchange inhibitor, Hoe 642: difference from preconditioning in the role of protein kinase C, J. Am. Coll. Cardiol. 29 (1997) 693–701.
[24] M. Buerke, H.J. Rupprecht, J. vonDahl, W. Terres, M. Seyfarth, H.P. Schultheiss, G. Richardt, F.H. Sheehan, H. Drexler, Sodium-hydrogen exchange inhibition: nover strategy to prevent myocardial injury following ischemia reperfusion, Am. J. Cardiol. 83 (1999) G19–G22.
[25] H. Serrar, P. Haddad, Effect of cold preservation and rewarming on rat liver cell volume regulation and concentrative amino acid uptake, Gastroenterology 112 (1997) 1344–1353.
[26] R. Carini, R. Autelli, G. Bellomo, E. Albano, Alterations of cell volume regulation in the development of hepatocyte necrosis, Exp. Cell Res. 248 (1999) 280–293.
[27] G.B. Rosser, G.J. Gores, Liver cell necrosis: cellular mechanisms and clinical implication, Gastroenterology 108 (1995) 252–275.
[28] R. Carini, G. Bellomo, M.U. Dianzani, E. Albano, Evidence for a sodium-dependent calcium influx in isolated rat hepatocytes undergoing ATP depletion, Biochem. Biophys. Res. Commun. 202 (1994) 360–366.
[29] R. Carini, M.G. DeCesaris, G. Bellomo, E. Albano, Role of Na^+/Ca^{2+} exchanger in preventing Na^+ overload and hepatocyte injury: opposite effects of extracellular and intracellular Ca^{2+} chelation, Biochem. Biophys. Res. Commun. 232 (1997) 107–110.
[30] W. Gao, Y. Takei, I. Marzi et al., Carolina rinse solution - A new strategy to increase survival time after orthotopic liver transplantation in the rat, Transplantation 52 (1991) 417–424.
[31] L. Sanchez-Urdazpal, G.J. Gores, J.J. Lemasters, R.G. Thurman, J.L. Steers, Carolina rinse solution decreases liver injury during clinical liver transplatation, Transplant. Proc. 25 (1993) 1574–1575.
[32] Q. Ting, A.L. Nieminen, B. Herman, J.J. Lemasters, Mitochondrial permeability transition in pH-dependent reperfusion injury to rat hepatocytes, Am. J. Physiol. 273 (1997) C1783–C1792.
[33] J.M. Weinberg, J.A. Davis, M. Abarzua, T. Fajan, Cytoprotective effects of glycine and glutathione against hypoxic injury to renal tubules, J. Clin. Invest. 80 (1987) 1446–1454.
[34] R.C. Dikson, S.F. Bronk, G.J. Gores, Glycine cytoprotection during lethal hepatocellular injury from adenosine triphosphate depletion, Gastroenterology 102 (1992) 2098–2107.
[35] D.C. Marsh, P.K. Vreugdenhil, V.E. Mack, F.O. Belzer,

J.H. Southard, Glycine protects hepatocytes from injury caused by anoxia, cold ischemia and mitochondrial inhibitors, but not injury caused by calcium ionophores or oxidative stress, Hepatology 17 (1993) 91–98.

[36] P. Schemmer, B.U. Bradford, H. Bunzendahl, J.A. Raleigh, J.J. Lemasters, R.G. Thurman, Intravenous glycine improves survival in rat liver transplantation, Am. J. Physiol. 276 (1999) G924–G932.

[37] G. DenButter, S.L. Lindell, R. Sumimoto, M.K. Schilling, J.K. Southard, F.O. Beltzer, Effect of glycine in dog and rat liver transplantation, Transplantation 56 (1993) 817–822.

[38] R. Carini, G. Bellomo, M.A. DeCesaris, E. Albano, Glycine protects against hepatocyte killing by KCN or hypoxia by preventing intracellular Na^+ overload in the rat, Hepatology 26 (1997) 107–112.